SOME ASPECTS OF THE WATER ECONOMIES OF NINE SPECIES OF ANURAN AMPHIBIANS

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2

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Table of Contents

SOME ASPECTS OF THE WATER ECONOMIES OF NINE SPECIES OF ANURAN AMPHIBIANS

Introduction

The science of animal ecology includes all aspects of the interactions which occur among animals and their environment. A field as broad as this must obviously make use of the techniques and knowledge of many other sciences. The consideration of physiology and behavior is necessary to the understanding of the reactions of animals to certain conditions of the enviornment. The responses of animals to certain environmental factors may be studied under the controlled conditions of the laboratory. These responses, and the physiological basis of these responses, provide information that is basic to the concept of the ecological niche.

It is the purpose to this thesis to examine some aspects of anuran water economy which might disclose some features of evolutionary specialization and provide information basic to the ecology of these animals. Amphibians seem to be relatively unsuited for terrestrial life. They always excrete a hypotonic urine and apparently lack integumentary modifications to conserve water. On dry land water is lost continously by evaporation. Even in saturated air a small loss of water occurs because the temperature of the animal is always higher than the surrounding air (Adolph, 1932). Different species of amphibians have been found to have widely varying habitat preferences with respect to the availability of water.

By examining the water economies of a number of anuran species which show differences in habitat preference it may be possible to better evaluate the specialization, if any, which has occurred in the direction of terrestrialism in this group. Such knowledge will add to our understanding of how animals are interacting with their environment.

Any geographical region in which such a study should be undertaken must show enough ecological variation to support anuran amphibians which exhibit species differences in habitat preference with regard to the availability of water. The Lake Itasca area in northwestern Minnesota is in the ecotone of the northern coniferous forest and the prairie, and provides a wide variety of habitats for anuran amphibians. The distribution of the primitive vegetation regions of Minnesota is illustrated in Figure 1. Nine species of frogs and toads are known from this area. Their habitat preferences range from the semi-arid prairie of the terrestrial-fossorial toad, Bufo hemiophrys, to the freshwater habitat of the highly aquatic mink frog, Rana septentrionalis. This area obviously has the habitat variability necessary to the different niche requirements of a number of anurans which exhibit apparent differences in their independence of water.

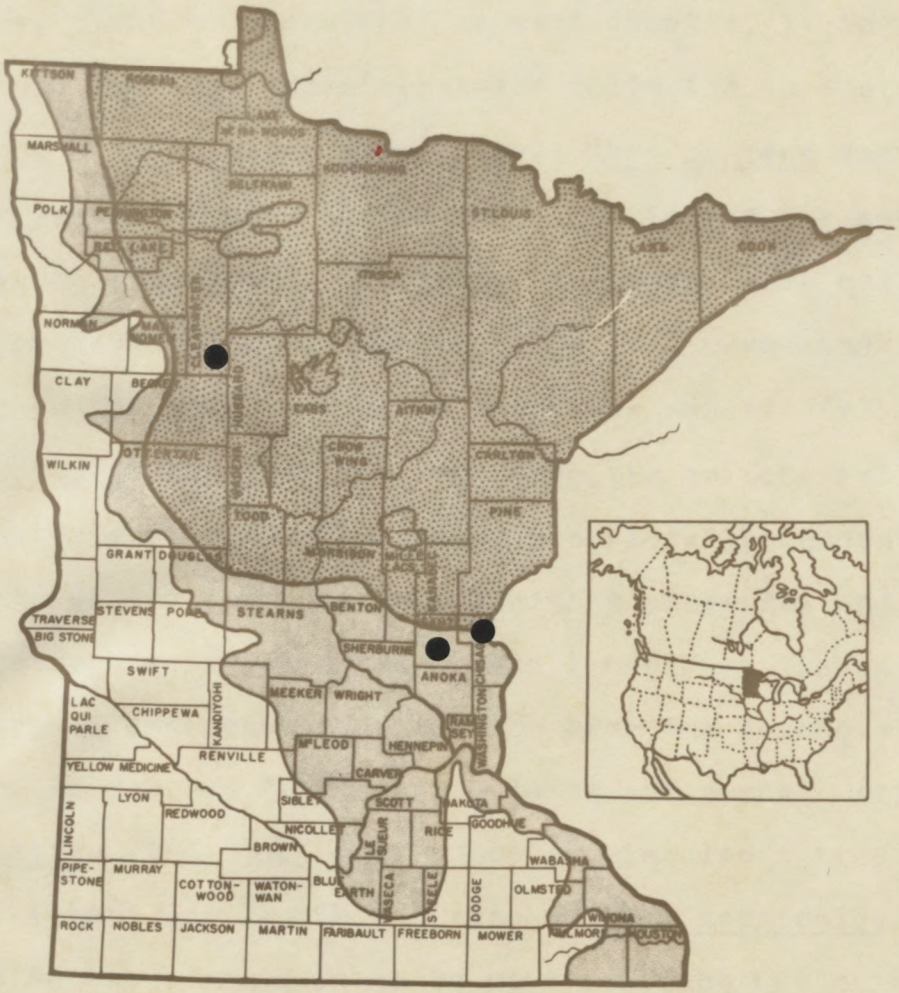
The habitat preferences of the nine species of frogs and toads includes in this study are summarized as follows. (1) Rana septentrionalis Baird, the mink frog, is probably the most aquatic of the species studied. It was never found far from permanent bodies of water. All specimens were collected on Lake Itasca in the water off

Figure 1. Distribution of the Primative Vegetation Regions of Minnesota.

● location of collection sites

Figure 1

PRIMATIVE VEGETATION REGIONS OF MINNESOTA



Coniferous Forest

Deciduous Forest

Prairie

(After Rosendahl and Butters, 1928)

floating bog shorelines. (ii) Rana clamitans Latreille, the green frog, is also a distinctly aquatic species, and like the mink frog, was rarely encountered far from water. This frog is rare in the Itasca area, and specimens for this study were collected from the St. Croix River near Never's dam. (iii) Rana sylvatica LeConte, the wood frog, might be classified as semi-aquatic, living in mature forests. Specimens were collected in deciduous woods of the Itasca area. (iv) Rana pipiens Schreber, the leopard frog, is also semi-aquatic and has successfully invaded a number of habitats. Specimens were collected in grassy fields, along the banks of streams and lakes, and in the brushy edges of deciduous woods. (v) Hyla versicolor LeConte, the tree frog, is an arboreal semi-aquatic species. It was only occasionally encountered on trees and shrubs in the vicinity of swamps and bogs. Specimens were collected in small ponds at Itasca where they had congregated during the breeding season. This frog is unknown from prairie lakes and ponds. (vi) Hyla crucifer Wied, the spring peeper, is also a tree frog, but about one fourth the size of Hyla versicolor. The spring peeper appears to be more restricted to moist bogs. Specimens were collected in sphagnum moss of tamarack and black spruce swamps. Although Itasca is well within the range of this species, they were not commonly encountered, and some of the specimens were collected near the Cedar Creek Natural History Area in Isanti County. (vii) Pseudacris nigrita LeConte, the swamp tree frog, is semi-aquatic, and is similar to Rana pipiens in that it has one

of the largest geographical distributions of the nine species studied. Specimens were collected in the same areas as the spring peeper. (viii) Bufo hemiophrys Cope, the Dakota toad, a terrestrial-fossorial species, is restricted to the prairie, and specimens were collected in Mahnomen County about forty-five miles west of Itasca. A recent study has disclosed that a great portion of the life of this animal is spent underground (Breckenridge and Tester, 1961). (ix) Bufo americanus Holbrook, the American toad, is also terrestrial-fossorial, but its range includes all habitat types present in Minnesota, although it is absent from the prairie of the northwestern part of the state. Specimens were collected in the vicinity of Itasca, usually along trails in deciduous woods. These brief descriptions of preferred habitats were drawn from field notes and from the published works of others (Wright and Wright, 1949: Oliver, 1955: Stebbins, 1954; and Breckenridge, 1944).

The results of a study by Marshall and Buell (1955) on the differences in habitat preferences of some of the anuran amphibians of the Itasca area are summarized in Table 1. They found no differences in the food habits of these species, and suggested that differences in microclimate or "escape facilities" of the four vegetation zones might be factors partially responsible for the observed differences in species distribution.

The importance of undertaking a comparative study of amphibian water economy in an area that supports a variety of species with different habitat preferences

Table 1. The occurrence of amphibians in the four vegetation zones of Twin Lakes Bog for the years 1949 through 1953.*

Species	Sedge Mat	Tamarack	Spruce	Fir-Ash
Mink Frog	2	3	0	0
Leopard Frog	256	61	8	5
Wood Frog	8	80	99	185
Spring Peeper	0	9	23	29
Swamp Tree Frog	0	3	28	44
Tree Frog	3	1	1	6
American Toad	0	0	0	1

*Numbers represent the total number of animals captured in each of the four vegetation zones for the five years of this study. After Marshall & Buell (1955).

becomes apparent when consideration is given to the possibility of physiological acclimation to environmental factors which may vary a great deal within the range of the species. Such factors might include temperature, precipitation, soil chemistry, and water chemistry. Previous workers who have relied upon biological supply houses for their animals were unable to evaluate the variation possibly induced by the unknown past history of their specimens. The past history includes the conditions in which the animals were held between collection and shipment. In this study it was possible to collect animals in the field and to assume that any differences in physiological acclimation which might have been present were due to the habitat variation with the area. Only in such a study can the niche requirements of difference species be

evaluated with regard to some environmental factor--in this study, the factor was water.

The research on which this theses is based includes comparisons among species of anuran amphibians of: (i) response to controlled desiccation stress, (ii) effective osmolarity[1] of the blood, (iii) permeability of the skin to water, (iv) active transport of sodium by the skin, and (v) response to a controlled hydration stress. The following section is a brief review of the literature concerning amphibian water economy.

[1]The effective osmolarity was expressed as the equivalent molarity of sodium chloride as measured by a melting point technique.

REVIEW OF THE LITERATURE

Early studies done to explain the apparent differences in dependence upon water, as indicated by differences in habitat preference, consisted of exposing amphibians to controlled stress of desiccation in laboratories and observing the responses of specimens in terms of (i) ability to withstand dehydration and (ii) rate of water loss. More recent studies have included analysis of such factors as (i) rate of water uptake after dehydration, (ii) adjustment of rate of water loss in response to dehydration, and (iii) the effects of endocrines on water balance.

The ability of amphibians to withstand loss of water has been found to be different for different species, and has been correlated with the ecology of these species. Hall (1922) found that Ambystoma punctatum and Rana pipiens died on losing about forty-seven and forty percent of their normal weight respectively. Thorson and Svihla (1943) showed that terrestrial species of anurans tolerated dehydration better than did species from aquatic habitats. The terrestrial toad, Scaphiopus holbrookii, and the aquatic frog, Rana grylio, died at losses of about sixty and thirty-eight percent of their body water respectively. Eight other species, with habitat preferences intermediate to S. holbrookii and R. grylio, showed tolerances to desiccation which fell within these limits. Littleford, Keller and Phillips (1947) found that for the semi-aquatic salamanders, Eurycea bilineata and Desmognathus fuscus, the vital limits of desiccation were similar, about fifteen

percent loss of body weight; however, the terrestrial salamander, Plethodon cinereus, tolerated a loss of about twenty-six percent. Cohen (1952) and Ray (1958), also working with salamanders, found an even greater range in tolerances to dehydration. Various species showed a tolerance to weight loss which varied from seven to fifty percent. The ability to survive water loss was again correlated with habitat preference; i.e., the greatest loss was tolerated by thosespecies which lived in the driest habitat. Ray (1958) further pointed out that differences in tolerance to water loss occurred at the familial, generic and species levels. Bently, Lee and Main (1958) found that species of frogs of the genera Neobatrachus and Heleioporus showed no correlation between ability to withstand dehydration and dryness of habitat. Thorson (1955) found for the terrestrial toad, Scaphiopus hammondii, and two frogs, the semi-aquatic Rana pipiens and the aquatic Rana clamitans, not only that the tolerance tended to be greater in small animals than in large ones, but that there was a correlation between tolerance and habitat preference--again the more terrestrial species were more tolerant to desiccation. The above studies suggest that an increased tolerance to desiccation is an adaptation of amphibians to life on land.

Rey (1937)found that the rate of cutaneous evaporation is approximately three milligrams of water per hour per square centimeter of surface in the four species which he studied. Thorson and Svihla (1943) did not observe differences in the rates of desiccation of the toad, S. holbrookii,

which lives in semi-arid regions, and of the aquatic frog, R. grylio. Furthermore, Overton (1904) had shown that at moderate rates of desiccation the loss of water from the skin of the frog is about the same as from the surface of free water. Thus rate of water loss has been suggested as being of no importance in terrestrialism of amphibians (Thorson, 1955).

Comparative experiments on the rate of water uptake through the skin have been performed by Rey (1937) on anurans and urodeles. At room temperature, the terrestrial species, Bufo vulgaris and Salamandra maculosa, were found to absorb respectively twenty-two and seventeen milligrams of water per square centimenter of surface per hour. The corresponding figure for R. temporaria was nine milligrams, and for the semi-aquatic R. esculenta, five milligrams. These results demonstrated a correlation between the rate of water uptake and the availability of water in the preferred habitats of different species of amphibians. The rate of water uptake tended to be greater in the more terrestrial species. Jorgensen (1949, 1950) found that cutaneous water uptake for the toad, B. bufo, was ten to thirteen mg./cm.^{2}/hr., and that for the frog, R. temporaria, was between six and seven mg./cm.^{2}/hr. Bently, Lee and Main (1958) showed that species of the genus Neobatrachus from dry areas took up water more rapidly than those from moist areas. They found no such correlation for species of the genus Heleioporus. Thorson (1955) found that species from aquatic habitats were able to recover from dehydration

more rapidly than species from terrestrial habitats. This, however, does not necessarily conflict with the previous results because species differences in body weight would affect the ratio of surface area to volume. The time to recover from dehydration would be a function of this ratio, whereas water flux per unit of surface area would not be a function of this ratio. It may be concluded from these studies that there is a tendency for water uptake to be more rapid in terrestrial species of amphibians. Differences in rate of water uptake might be related to species differences in such factors as (i) skin permeability to water, (ii) osmolarity of the blood and (iii) response to antidiuretic hormone (ADH).

Injection of mammalian neurohypophyseal extract causes an increase in the body weight of amphibians if they are kept in water. Steggerda (1937) compared the reaction to pituitrin of a toad, two species of frogs and a salmander, all animals receiving ten units per hundred grams body weight. The toad, B. americanus, responded with a weight increase of about forty-five percent, whereas the two frogs, R. pipiens and R. clamitans, gained eighteen and fourteen percent respectively. The aquatic salamander, Necturus maculosus, gained only four percent in weight. These results led to the conclusion that a strong reaction to pituitrin was related to adaptation of amphibians to life on land. Jorgensen (1950) compared the antidiuretic effects of mammalian neurophypophyseal extract on the frog, R. temporaria, and the toad, B. bufo, and found that in terms of cutaneous water uptake,

grams of water per hour, the effect on the toad was ten times greater than on the frog. Also an extract of the neurohypophysis of the frog was compared with an extract of toad gland with regard to induction of antidiuresis. Injections of frog gland extract into frogs and toads caused no observable antidiuresis, whereas the toad gland extract was antidiuretically active in both frogs and toads. When in physical contact with water, terrestrial species seem to be able to replace a water deficiency at a higher rate that aquatic species. This may be of importance where free water is only temporarily available, and the water balance hormone, ADH, seems to be of importance as a regulative mechanism.

Osmolar concentration of the body fluid is another factor which could elicit differences in the rate of hydration. The significance of such a mechanism would be twofold. For aquatic species, the rate at which water would enter the body could be reduced due to the lower concentration gradient between body fluids and the surrounding water. On the other hand, the rate of rehydration for terrestrial species could be enhanced. Prosser, et. al., (1950) reported that the freezing point depression of the blood of a frog, Rana sp. was less than that of a toad, Bufo sp. Tercafs and Schoffeniels (1962) reported a value for the freezing point depression of the toad, B. viridis, which was very close to the value reported by Prosser et. al. for a toad. These data indicated that differences in osmolarity of body fluids might occur among different species of amphibians. Such differences could be

of adaptive significance to terrestrialism in this group.

There have been a number of studies of the permeability of amphibian skin (Maxwell, 1913; Adolph, 1925, 1930, 1931, 1933; Krogh, 1937, 1938, 1939; Overton, 1904; Ussing, 1954; MacRobbie and Ussing, 1962). These workers studied the movement of water and inorganic ions through intact and isolated amphibian skin. The movement of water was found to be rapid relative to the flux of inorganic ions. Comparative studies describing differences in skin permeability are lacking. Such studies might determine whether or not differences in the permeability of skin to water could be of adaptive value to species which exhibit differences in habitat preference in regard to the availability of water.

Thorson (1956) described an "adjustment of water loss in response to desiccation" for three species of anurans. Specimens were exposed to repeated dehydrations and examined for changes in rates of water loss. When desiccation was followed by rehydration and repeated desiccation, the subsequent rate of water loss tended to be lower. This adjustment of the rate of water loss would appear to have adaptive value in terrestrial habitats.

Those species of anurans which spend most of their lives in water; e. g., R. septentrionalis and R. clamitans, are under the continous stress of hydration. Water is always entering the body by passive transport and a hypotonic urine is produced to reduce the dilution of body fluids. Although the urine is dilute, some salts are

lost. The skin of amphibians has been shown to actively remove sodium from surrounding freshwater to replace the lost salts (Huf, 1936; Krogh, 1937, 1938; Ussing, 1949). Ussing and Zerahn (1951) developed the "short-circuited" frog skin preparation for measuring the rate of sodium transport. This electrical model has been validated by the use of sodium isotopes, and has proven useful in the study of active transport. The effects of antidiuretics on this system have been studied by Linderholm (1952), Anderson and Ussing (1957) and Kalman and Ussing (1955). In general it has been found that the skin of terrestrial species showed a greater response to ADH, in terms of changes in potential difference and rate of sodium transport, than the skin of aquatic species. Comparisons of the rate of sodium transport among different species have not been made. Such a study might show specialization to aquatic habitat through natural selection for an increased rate of transport associated with constant water turnover.

The effects of stress of hydration might be experimentally measured in the laboratory by subjecting specimens to sustained immersion in distilled water. No interspecific comparisons of this nature have been made. Such a study might demonstrate that not only an adaptation of aquatic species to life in water has occurred, but that terrestrial species cannot tolerate prolonged periods in water: i. e., specialization to preferred habitats. This concludes the literature review.

Five sections, each of which is a separate area of

experimental procedure are presented next in the following order: (i) response to controlled desiccation stress, (ii) effective osmolarity of blood plasma, (iii) permeability of skin to water, (iv) active transport of sodium by the skin, and (v) response to controlled hydration stress.

The Responses of Nine Species of Anuran Amphibians to Controlled Desiccation Stress

Materials and Methods

Field collections were made during the summers of 1960 and 1961 in several different habitat types in the vicinity of the biological station of the University of Minnesota at Lake Itasca, Clearwater County, Minnesota. These habitats included cattail-sedge marshes, freshwater lakes and ponds, spruce and tamarack bogs, upland coniferous and deciduous woods, and grasslands. One hundred and fifteen specimens of eight species, R. pipiens, R. sylvatica, R. septentrionalis, Hyla versicolor, H. crucifer, Pseudacris nigrita, B. americanus and B. hemiophrys, were collected in this area. During the summer of 1961 eight specimens of P. nigrita and H. crucifer were taken near the Cedar Creek Natural History Area, Isanti County, twenty specimens of R. pipiens from Lake Francis, Isanti County, and ten specimens of R. clamitans from the St. Croix River in the vicinity of Never's Dam, Chisago County. One hundred fifty-three specimens were collected for this study. The solid black circles in Figure 1 represent the locations of the collection sites.

The specimens were held in half-gallon polyethylene jars for at least three days and not longer than six days before they were placed in the desiccation chamber. About one fourth of an inch of water and pieces of sphagnum moss were kept in these jars. The animals were not fed

during this holding period. Before an animal was placed in the desiccation chamber, it was thoroughly rinsed, dried in paper toweling, weighed and measured. Specimens whose weight was five grams or more were place in a glass weighing bottle and weighed on a triple beam balance. Specimens which appeared to weigh less than five grams were weighed on an analytical balance. Body length was measured from the tip of the snout to the distal end of the urostyle.

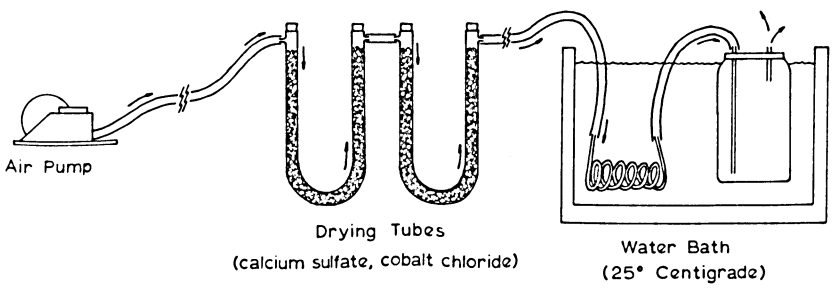
The desiccation chamber was a modification of that first used by Hall (1922). It was essentially a pint mason jar with two sections of copper tubing soldered to the lid for inlet and outlet of air. The air source was a Marco Model B electric pump manufactured by the J. B. Maris Company, Bloomfield, New Jersey. This unit was capable of pumping air at the rate of 700 cubic centimeters per minute. The air was passed through two drying tubes which contained calcium sulfate and cobalt chloride, and seven feet of one-eighth diameter copper tubing before it entered the desiccation chamber. The calcium sulfate removed moisture from the air and the copper tubing, submerged in a water bath at 25° Celsius, equilibrated the air to that temperature (Figures 2 and 3). This setup provided a relatively constant environment for the specimens while they were being dehydrated: air flow was 700 ± 10 cubic centimeters per minute, temperature was constant at 25° C. and the relative humidity approached zero percent.

Figure 2. Setup for desiccation experiments.

Figure 3. Desiccation chamber.

A-Copper tubing
B-Rubber tubing
C-Glass Jar (1 pint)

Figure 2. Setup For Desiccation Experiments



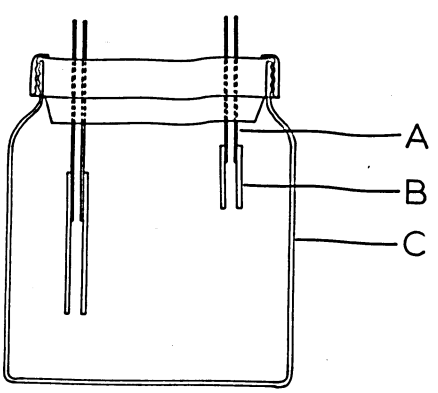


Figure 3. Desiccation Chamber

The specimens were placed in the desiccation chamber under a constant stress of dryness, and dehydrated to a point from which they could not recover if placed in moist paper toweling. The "point of no return" occurred between the time at which the specimen lost its ability to right itself when upset, and the time at which it was "dead" as indicated by darkening of the skin and complete loss of limb movement. Then, the animal was reweighed and placed in an oven at 98° C. and dried to a constant weight. This dry weight was assumed to be the weight of the specimen after the loss of one hundred percent of its body water.

The validity of using weight loss as a measure of loss of body water was checked by three different techniques.(i) The air leaving the drying chamber was periodically run through drying tubes. The weight gain of the desiccant in the drying tubes was approximately equal to the weight loss of the specimen. (ii) Specimens which were dehydrated for a short time until approximately fifteen percent of total body water had been lost were observed to return to their original weight when placed in water. (iii) Animals kept in the desiccation chamber for forty hours with one fourth of an inch of water and sphagnum moss did not lose weight. These results indicate that loss of weight is a valid estimate of loss of body water under the conditions of this experiment.

Results

The vital limit of water loss (tolerance to desiccation) for each species is shown on Figure 4 and Table 2. The vital limit of water loss is expressed as the percent of water lost before death. The latter statistic was calculated for each speciman by dividing the amount of water (grams) lost before death by the total water (grams) and multiplying this quotient by one hundred. Total water was calculated by subtracting dry weight from fresh weight. The results are arranged by species in order of those of the most aquatic habitats to the most terrestrial. The mean values of tolerance to desiccation, expressed as the percent of water lost before death, ranged from 35.1 for the aquatic mink frog, R. septentrionalis, to 58.0 for the spring peeper, P. nigrita.

The rate of water loss is inversely related to the size of the specimen (Figure 5). If the rate of desiccation has influenced the tolerance to desiccation, there should be a negative correlation between body weight and tolerance. The correlations between weight and tolerance for each species are presented in Table 4.

Discussion

The existence of a greater tolerance to water loss in terrestrial species than in aquatic species has been described in a number of previous investigations

Figure 4. Tolerance to desiccation.

The following symbols will be used to represent the estimate of variation about the mean of each species in this study with the exception of Figure 11.

Species mean ± 2 Standard Errors

Species mean ± 2 Standard Deviations

Figure 4

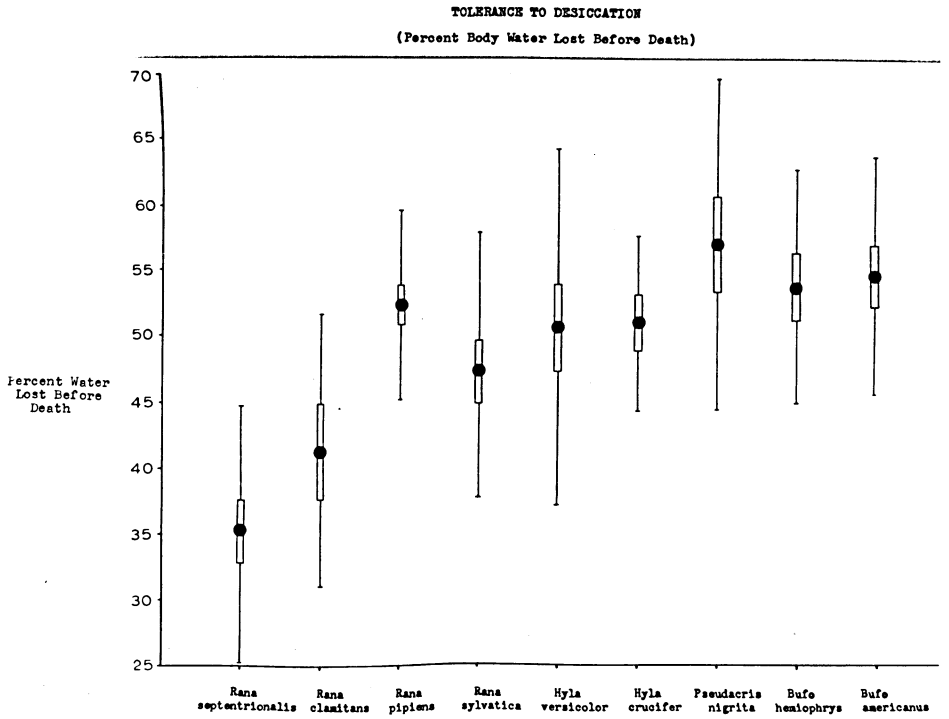


Table 2

TOLERANCE TO DESICCATION

(Percent Body Water Lost Before Death)

Species	Mean	Standard Deviation	Standard Error of the Mean	Coefficient of Variation	Number of Specimens
Rana septentrionalis	35.1	5.12	1.14	.146	20
Rana clamitans	41.5	5.21	1.65	.125	10
Rana pipiens	52.2	4.06	.69	.078	35
Rana sylvatica	47.1	5.42	1.31	.115	17
Hyla versicolor	50.3	7.21	1.80	.143	16
Hyla crucifer	50.6	3.67	1.16	.073	10
Pseudacris nigrita	58.0	6.46	2.04	.111	10
Bufo hemiophrys	53.4	4.36	1.38	.082	10
Bufo americanus	54.4	4.11	1.30	.076	10

(Overton, 1904; Hall, 1922; Bey, 1937; Thorson and Svihla, 1943; Littleford, et. al., 1947; Thorson, 1955; and Bay, 1958). In few of these studies were the experimental animals freshly collected in the field or from the same geographic area. The present results indicate a tendency for terrestrial species to be more tolerant of desiccation, which is in agreement with previous studies. The mean tolerance to desiccation of two species of highly aquatic frogs, R. septentrionalis and R. clamitans, is significantly different (P= .05) from the mean tolerance of the rest of the species which are more terrestrial in their habitat preferences.

Thorson (1955) has suggested that pronounced differences existed in tolerance to water loss among individuals of the same species and that these differences were inversely related to the size of the animal. The results of the present study have demonstrated that tolerance to desiccation does not appear to be related to the size of the specimen. The correlation coefficients between size and tolerance are neither consistent with respect to sign or magnitude, nor are they significantly different from zero, with the exception of one. The total correlation coefficient, representing the pooled data of Table 4 is not significantly different from zero. The value of the total correlation coefficient is .089 for one hundred thirty-five specimens.

The rate of water loss, expressed as percentage loss per hour, varies intraspecifically depending on the weight of the specimen. The decrease in rate appears

Table 3

PERCENT BODY WATER

Species	Mean*	Standard Deviation	Standard Error of the Mean	Coefficient of Variation	Number of Specimens
Rana septentrionalis	80.0	2.31	.517	.0289	20
Rana clamitans	83.1	1.52	.482	.0183	10
Rana pipiens	83.5	2.59	.438	.0310	35
Rana sylvatica	81.4	2.42	.587	.0297	17
Hyla versicolor	80.3	3.38	.795	.0421	18
Hyla crucifer	79.9	2.31	.732	.0289	10
Pseudacris nigrita	77.4	3.92	1.240	.0506	10
Bufo hemiophrys	80.5	3.33	1.005	.0413	11
Bufo americanus	81.8	1.95	.617	.0238	10

* Body water expressed as percent of total body weight

Table 4

CORRELATION BETWEEN BODY WEIGHT AND TOLERANCE TO DESICCATION

Species	BODY WEIGHT		TOLERANCE TO DESICCATION		CORRELATION COEFFICIENT (r)	N
	Mean	Standard Deviation	Mean	Standard Deviation		
Rana septentrionalis	11.7	4.76	35.1	5.12	.100	20
Rana clamitans	6.9	3.74	41.5	5.21	.323	10
Rana pipiens	10.4	7.67	52.2	4.06	.152	35
Rana sylvatica	3.6	2.87	47.2	5.42	-.226	15
Hyla versicolor	4.2	2.57	50.0	7.21	.032	15
Hyla crucifer	0.66	0.24	50.6	3.67	-.203	10
Pseudacris nigrita	0.65	0.13	58.0	6.46	.797**	10
Bufo hemiophrys	3.9	3.23	53.4	4.36	-.372	10
Bufo americanus	6.4	5.81	54.4	4.11	.524	10

** Significantly different from zero with probability, P = .99
No other r values were significantly different from zero at P = .95

N indicates the number of specimens

to be monotonic, but not constant, for a constant increase in weight (Figure 5). Thorson (1955) described a similar relationship between these variables, but offered no explanation. Overton (1904), Rey (1937) and Thorson and Svihla (1943) have demonstrated that under conditions of constant humidity, temperature and air flow, the absolute rate of water loss (grams of water per unit time) is proportional to the surface area of the specimen and is approximately the same for different species of amphibians. Adolph (1933), Rey (1937) and Krogh (1939) suggested that $(\text{weight})^{2/3}$ could be used as an index to surface area. By assuming that water, expressed as percent of total body weight, does not vary a great deal among species (See Table 3), it is possible to use weight as an index of total water. The results of the above studies are used as evidence for the following relationships.

(i) $\text{rate} = \dfrac{\text{grams of water lost per unit time}}{\text{total water (grams)}} =$

$=$ percent water lost per unit time

(ii) $\dfrac{\text{grams of water lost}}{\text{unit time}} = K\ (\text{surface area})$

(iii) $(\text{weight})^{2/3} = K\ (\text{surface area})$

(iv) $\text{weight} = K\ (\text{total water})$

(v) $\text{rate} = K\ \dfrac{(\text{weight})^{2/3}}{\text{weight}} = K\ (\text{weight})^{-1/3}$

Thus Figure 5 might be interpreted as $(\text{weight})^{-1/3}$ plotted against (weight). A straight line relationship between the two variables, rate and weight, should be obtained when the coordinates of Figure 5 are transformed

Figure 5. Relationship between weight and rate of loss of body water.

The data represent 135 different animals belonging to nine different species.

Figure 6. Relationship between log (weight) and log (rate of loss of body water).

Figure 5

Relationship Between Weight And Rate Of Loss Of Body Water

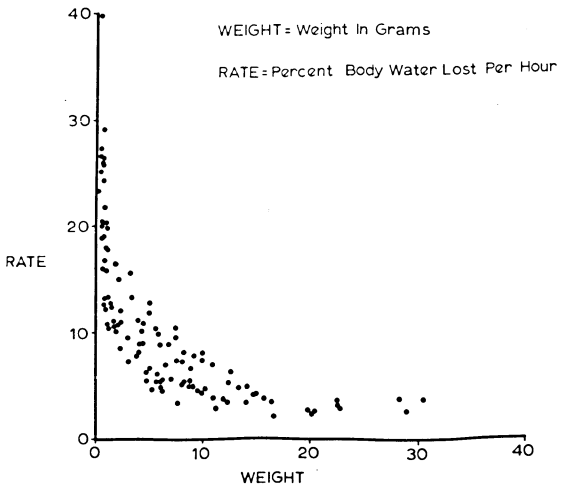
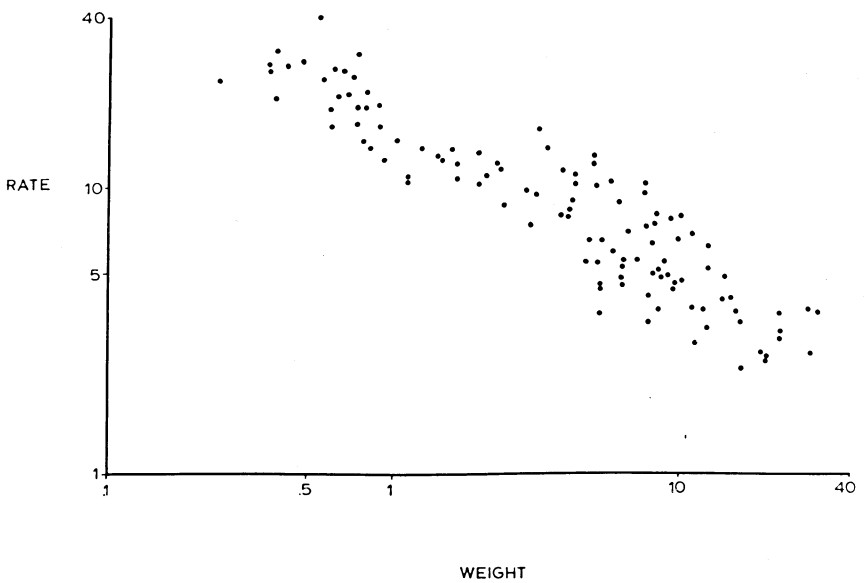


Figure 6



to logarithmic scales, and the slope should be approximately -.33. Figure 6 is a graph of log (rate) plotted against log (weight) of the data of the present study. The slope of the regression of log (rate) on log (weight) is -.43, which is not significantly different from the expected slope of -.33. The standard error of the slope is equal to .061. The expected slope is a function of the use of weight $^{2/3}$ as an index to total surface area. In this study, where the entire surface of the specimen was not exposed, such an index might not be as appropriate as some other power function of weight. Thus these results, and those of previous workers, are consistent. The relationship between weight and rate of water loss (percentage) appears to be the result of different surface area to volume ratios for specimens of different weights.

Another important consideration of the relationship between the rate of water loss (percentage) and weight is the fact that small animals lose water at a much faster rate than larger specimens because of an increase in the surface area to volume ratio as the size of the animal decreases. This means that species of small size might be at a distinct disadvantage because of the increased rate of water loss. An increase in body size would appear to be of adaptive value for those species of anurans which are found in dry habitats. However, this is contrary to fact since one of the smallest amphibian species studied, P. nigrita, is found in a relatively dry prairie habitat. The two species which show the strongest preference to aquatic habitat,

R. septentrionalis and R. clamitans, spend two seasons in the tadpole stage which results in young frogs of larger size. The apparent contradiction and the fact that the absolute rate of water loss is approximately the same for different species support the previous statement by Thorson and Svihla (1943), that adaptation to terrestrial life by amphibians has occurred primarily through the development of an ability to survive a greater loss of body water. However, the data of the present study or of any previous study on amphibian water economy do not warrant the suggestion that the rate of water loss is not of adaptive value to species living in more arid habitats.

These data must be taken for what they are. The experimental ecologist who studies interspecific response to a single factor, isolated and controlled in the laboratory, must realize that although his world is real, it is not necessarily natural. The experimental animal is a product of natural selection in an environment which imposes the influence of a multitude of factors (moisture, temperature, wind, food, competition, shelter, predation, etc.). It is the possible interaction of these factors which must be considered in the interpretation of an animal's response to the controlled conditions of a laboratory experiment. When experimental data appear to be inconsistent with the facts of known natural history, it would be best to reevaluate in terms of (1) contrasting laboratory conditions with those of the

environment, and (ii) considering the possibility of factor interaction.

The possibility of factor interaction certainly seems to be appropriate to the interpretation of the data of the rates of desiccation. The rate of loss of water, which appeared to be a function of body size, and independent of species differences, has been interpreted as being unimportant to the terrestrialism of amphibians by Thorson (1955). However, the maintenance of water loss(by evaporation) could be of primary importance to temperature control, especially for those species which live in drier habitats (Kirk and Hogben, 1946). Although this interpretation is completely teleological and has been presented without any experimental evidence, it is nevertheless a consideration which should be made in the final evaluation of these data.

Effective Osmolarity of Blood Plasma

Materials and Methods

Field collections were made in the summer of 1961 in the vicinity of the biological station at Lake Itasca. Fourteen specimens of four species, R. septentrionalis, R. pipiens, R. sylvatica, and R. americanus, were obtained. Two specimens of R. clamitans were collected near Never's Dam on the St. Croix River.

Specimens were held in half-gallon jars for no longer than four days before blood was removed to be analyzed. About one-fourth of an inch of water and pieces of sphagnum moss were kept in the jars. The animals were not fed.

The pericardial cavity of each specimen was exposed by a ventral incision and approximately one half cubic centimeter of blood was drawn from the heart with a 1 cc. tuberculin syringe fitted with a 22-guage needle. The blood was immediately transferred to a centrifuge tube. The blood cells were separated from the plasma by centrifugation at 1600 g for three minutes.

The technique used to determine the effective osmolarity of the plasma was based on a method described by Gross (1954). Small samples of plasma were drawn into three-centimeter sections of thin-walled capillary tubing (1 mm. I.D.) and the ends were plugged with vaseline. An air space was left on each side of the sample so that the vaseline was not in contact with the plasma. After the samples were frozen at minus twenty

degrees Celsius. They were transferred to a solution of twenty-five percent alcohol which had been chilled to about -5° C. The bath was agitated by a stirrer and allowed to warm slowly, at the rate of 1° C. per forty minutes. The frozen samples were viewed by transmitted light between "crossed" Polaroid sheets. The ice crystals were observed as white against a deep blue background. As the bath warmed, the samples melted in sequence according to their effective osmolar concentrations. The endpoint in melting was arbitrarily set at one half of the volume of the sample rather than at complete thawing to avoid the possibility of confusion due to minute crystals of pure water persisting after the melting point of the solution had passed.

The effective osmolarities of the samples were obtained by interpolation on a curve constructed by plotting the osmolarities of standard solutions against the times of melting. The standards consisted of aqueous solutions of .12, .14, .16, .18, .20 and .22 molar sodium chloride. Two samples were taken from each specimen and two melting point determinations were done for each sample.

Results

The effective osmolarities of the blood plasma of five species of anurans are presented in Figure 7 and Table 5. The results are presented by species, arranged in order from those of aquatic habitat to those of more terrestrial habitat. These data indicate a tendency for the aquatic species to have a lower effective osmolarity of plasma than terrestrial species. The mean values of

Figure 7

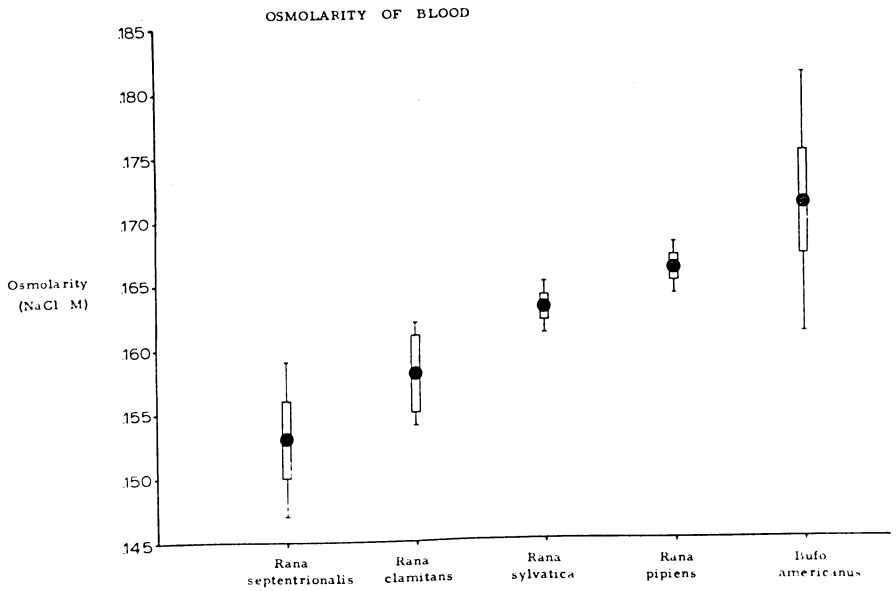


Table 5

OSMOLARITY OF BLOOD (NaCl M)

Species	Mean	Standard Deviation	Standard Error of the Mean	Coefficient of Variation	Number of Specimens
Rana septentrionalis	.153	.003	.002	.0196	4
Rana clamitans	.158	.002	.002	.0127	2
Rana sylvatica	.163	.001	.0007	.0060	3
Rana pipiens	.166	.001	.0007	.0060	3
Bufo americanus	.171	.005	.002	.0292	5

plasma concentration, expressed as molarity of sodium chloride, ranged from .153 M for the aquatic frog, R. septentrionalis, to .171 M for the terrestrial toad, B. americanus. The F value for interspecific variation from the analysis of variance of these data (Table 6) was significant (P = .05). Also the mean values of effective osmolarity of the plasma of the two most aquatic species, R. septentrionalis and R. clamitans, was significantly different (P = .05) from the mean of the most terrestrial species sampled, B. americanus. The F value for variation

Table 6. Analysis of Variance. Effective osmolarities of the blood plasma of five species of anurans.

Source	D F	M S	$F_{obs.}$	$F_{.95}$
Species	4	51.1	3.55	3.36
Animals/Spp.	11	14.4		
Samples/Anim./Spp.	16	2.1	1.14	2.01
Determinations/Samp./Anim./Spp.	32	1.8		

DF indicades the degrees of freedom.
MS indicates the mean sum of squares.
The values given for the mean sum of squares have been multiplied by 10^6.

between samples/animals/species from the above ANOVA was not significant. A significant F value would have indicated that the sampling technique had introduced a significant amount of variation relative to the precision of the technique of analysis.

Discussion

The mean values of the effective osmolariy of plasma, expressed as molarities of sodium chloride, ranged from

.153 M for R. septentrionalis to .171 M for B. americanus. The freezing point depression values which correspond to these mean values of osmolarity are -.55° C. for R. septentrionalis and -.63° C. for B. americanus under assumed conditions of standard temperature and pressure. These data are similar to the results reported by Prosser et. al. (1950) The freezing point depression for Rana spp. was reported to be -.56 and that for Bufo spp. was -.76° C. Tercafs and Schoffeniels (1962) reported a value of -.73° C. for the freezing point depression of the blood of B. viridis. The results of the present study are similar to the results of previous workers.

The above results demonstrate a tendency for aquatic species to have lower osmolar concentration of blood plasma than terrestrial species. The increasing effective osmolarity of the blood of the more terrestrial species might tend to reduce the rate of water loss through the skin by evaporation because of the lowering of the vapor pressure. However the observed differences in concentration of blood plasma do not seem to be significant in this regard since interspecific differences in rates of water loss have been shown to be insignificant (Rey, 1937; Thorson and Svihla, 1943).

A lower osmolar concentration of plasma would tend to decrease the rate at which water could enter an animal by passive transport because of the reduced concentration gradient of water between the body fluids and the surrounding freshwater. This would seem to be of adaptive

value to species of anurans which spend most of their lives in water. The higher osmolarities of plasma (and body fluids) of the more terrestrial species would tend to increase the rate at which water could enter the body by passive transport because of the increased concentration gradient of water. This could be of adaptive value to terrestrial species which are not in continuous contact with water because of the potential for an increased rate of water replacement. The occurrence of an increasing osmolarity of blood plasma from aquatic is not only consistent with the results of Rey (1937), Jorgensen (1949, 1950) and Bently, Lee and Main (1958) which demonstrated that terrestrial species absorbed water at greater rates than did aquatic species, but suggests a functional relationship. The regulation of passive water uptake may have developed by natural selection for higher or lower osmolarities of blood and body fluids during the evolutionary specialization of amphibians to life in different habitats. Differences in the permeability of skin to water is another factor which may be functionally related to differences in rates of absorbtion of water among species of anurans.

Permeability of Skin to Water

Materials and Methods

Thirteen specimens of five species, R. septentrionalis, R. pipiens, H. versicolor, B. hemiophrys and B. americanus, during the summer of 1961 in the vicinity of Itasca Lake. Specimens were kept in half-gallon polyethylene jars for no longer than seven days. About one fourth of an inch of water and pieces of sphagnum moss were kept in the jars. The animals were not fed.

The method used to measure the permeability of amphibian skin to water was based on a technique described by Maxwell (1913). A bag was made from the skin of each hind leg. The skin was cut around the circumference of the thigh and gently rolled down the leg and over the foot. An inverted tube was obtained by cutting the skin off at the ankle. The tube of skin was rinsed in Ringer's solution and one end was tied shut with carpet thread. One milliliter of diluted Ringer's[1] was placed in one bag and undiluted Ringer's in the other bag. The remaining open end of each bag was then tied shut. These bags, with the dermal surface of the skin to the outside, were placed in Ringer's solution for another rinsing. After ten minutes they were removed and the excess moisture was drained from the surface by dragging them across the lip of a beaker. The bags were weighed twice on a Roller-Smith

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[1] Diluted Ringer's was made by mixing four parts of distilled water with one part Ringer's.

precision balance and placed in separate beakers with one hundred milliliters of aerated Ringer's solution. After two hours they were removed and reweighed. The bags were then opened, cut lengthwise and unrolled on a sheet of graph paper. The outline of the bag was traced and used as a measure of skin area.

The movement of water through the skin of each bag could be calculated by assuming that the loss of weight was due to the movement of water from the inside of the bag into the surrounding solution. Water leaving the bag which contained diluted Ringer's was moving passively down a concentration gradient. The other bag which contained Ringer's served as a control. Huf, Doss and Wills (1957) and Kirshner, Maxwell and Fleming (1960) described a "non-osmotic movement" of water in isolated frog skin preparations. They suggested that this movement of water was related to the active transport of ions by the skin. The index of permeability to water for the skin of a given specimen was obtained by subtracting the weight loss/hour, cm.^{2} of the control bag from the weight loss/hour, cm.^{2} of the experimental bag.

Results

The indices of skin permeability to water for five species are presented in Figure 8 and Table 7. The results are presented by species, arranged in order from those of more aquatic habitat to those of more terrestrial habitat. These data show a tendency for the skin of the more aquatic species to have a lower permeability to water. The mean values of passive water transport, expressed as grams of

Figure 8

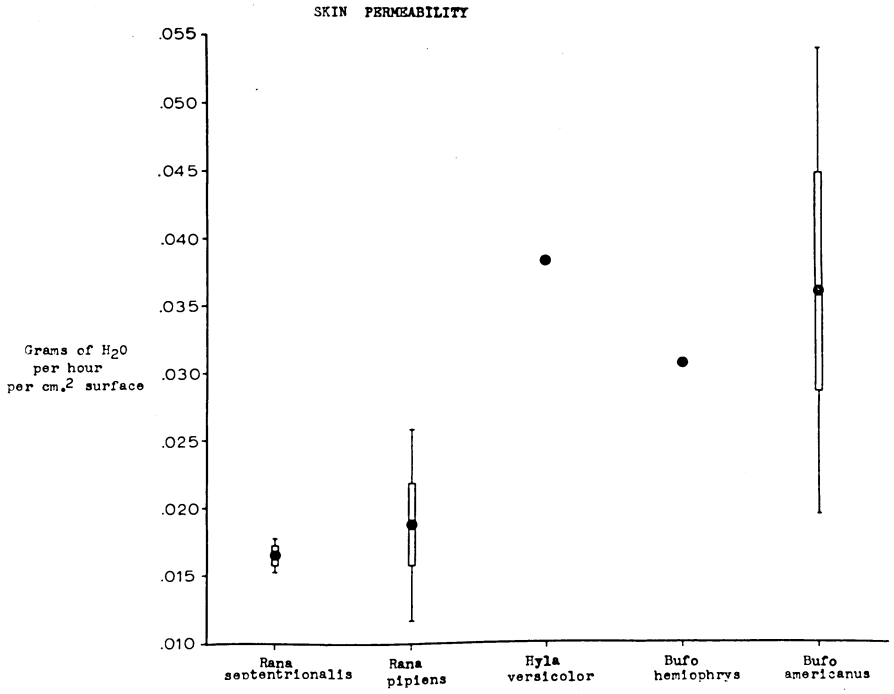


Table 7

SKIN PERMEABILITY

Species	Mean *	Standard Deviation	Standard Error of the Mean	Coefficient of Variation	Number of Specimens
Rana septentrionalis	.0165	.0004	.0003	.024	2
Rana pipiens	.0188	.0034	.0017	.181	4
Hyla versicolor	.0378				1
Bufo hemiophrys	.0305				1
Bufo americanus	.0366	.0085	.0039	.232	5

* Skin permeability expressed as grams of water moving through one square centimeter in one hour while concentration gradient is constant.

water per hour per square centimeter of skin surface, ranged from 0.0165 for the aquatic mink frog, R. septentrionalis, to 0.0366 for the more terrestrial American toad, B. americanus. The mean values of passive water transport for R. septentrionalis and R. pipiens were both significantly different (P = .05) from the mean value for B. americanus, but were not significantly different from each other.

Discussion

The results of this study suggest that there is a tendency for the skin of aquatic species to have a lower permeability to water than the skin of more terrestrial species. A lower permeability to water would tend to reduce the rate at which water could enter an animal living in an aquatic environment. This would seem to be of adaptive value to species which live continuously in water because of the reduction in energy expenditure necessary to higher rates of water turnover associated with excessive hydration. A greater permeability of skin to water would induce an increased rate at which water could enter the body of amphibian species which are only on temporary contact with water. The potential for an increased rate of water replacement would seem to have adaptive value for the more terrestrial species.

The differences in the permeability of skin of different species to water seem to augment the specialization to the availability of water in their preferred habitats which was previously demonstrated by the interspecific

variation in the osmolar concentration of blood plasma. Interspecific variation in these two factors, osmolarity of blood plasma and skin permeability to water, appear to be part of the physiological basis for differences in rates of rehydration previously described by Rey (1937), Jorgensen (1949, 1950) and Bently, Lee and Main (1958). Tolerance to hydration stress may be related to interspecific differences in osmolarity and water permeability. These relationships will be discussed in a following section.

Active Sodium Transport by the Skin

Materials and Methods

Sixty-nine specimens of nine species, R. clamitans, R. septentrionalis, R. pipiens, R. sylvatica, H. versicolor, P. nigrita, B. hemiophrys and B. americanus, were collected during the summer of 1961 in the vicinity of Lake Itasca. The specimens were kept in half-gallon polyethylene jars for no longer than ten days. About one fourth of an inch of water and pieces of sphagnum moss were kept in the jars. The animals were not fed.

The technique used to measure the rate of active sodium transport is based on the method described by Ussing and Zerahn (1951). Pieces of skin from the ventral and dorsal surfaces of a specimen were removed and placed in a 50 ml. beaker with well-aerated Ringer's solution. One of these pieces of skin was clamped between two halves of a plexiglass chamber so that an area of 0.50 $cm.^2$ surface was exposed. Both halves were immediately filled with Ringer's. Diagrams of the apparatus used in this experiment are shown in Figures 9 and 10. The potential difference between the outer and inner surface of the skin was recorded at five minute intervals until it reached an "equilibrium" value. When the potential difference reached some relatively constant value; e.g., ± 4 mV for 15 minutes, it was recorded as the "equilibrium" value. This usually occurred twenty to thirty minutes after the skin had been clamped in the chamber. An external source was used to buck out the equilibrium potential. The current (microamps.) that

Figure 9

Circuit Diagram For Short-Circuited Frog Skin

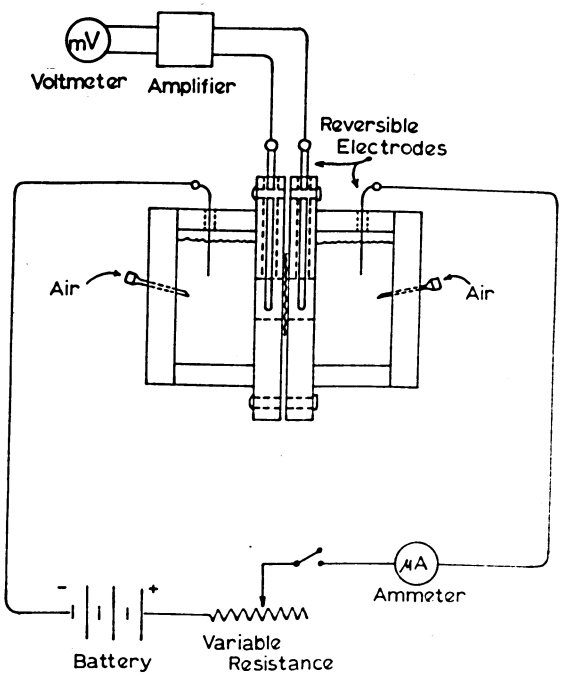
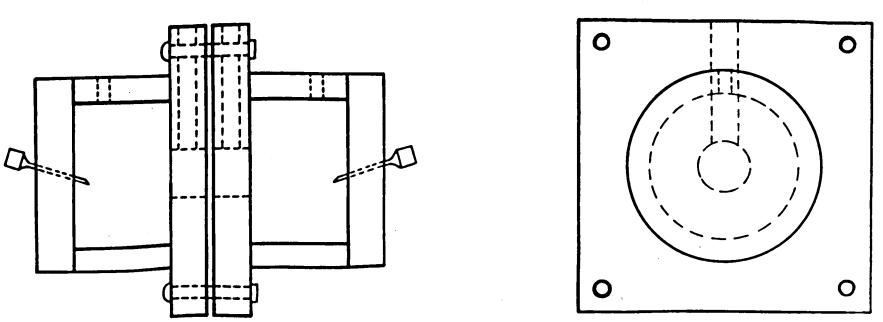


Figure 10

Chamber For Short-Circuited Frog Skin
(Plexiglass Construction)



Scale: ⟵½"⟶

was required to establish the null condition was recorded. This current[1] has been shown to be proportional to the net flux of sodium ions from the outer to the inner surface of the epithelium (Ussing and Zerahn, 1951). The current may be used as an index to the rate of transport of sodium by isolated pieces of skin from different species of anuran amphibians. However the potential difference which occurs across the skin in this preparation has been shown to influence the active transport of sodium ions (Zerahn, 1956). Therefore the current (μamps) was divided by the equilibrium potential (mvolts) to obtain an "adjusted" index to sodium flux (mhos x 10^{-3}) which was independent of differences in potential.

Results

The indices to the net rate of sodium transport through the skin of nine species of anurans are presented in Figure 11 and Table 8. The results are presented by species, arranged in order from those of more aquatic habitats to those of more terrestrial habitats. There were no apparent differences among the rates of transport across the skins of species from different habitats. This was true for comparisons of the current (μ amps) and the adjusted index, conductance (mhos x 10^{-3}).

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[1]The current, microampheres, necessary to reduce the potential difference to zero, divided by Faraday's constant, 96500 coulombs, is equal to the net sodium transport through the skin expressed as microequivalents of sodium per second per unit of surface area.

Figure 11. Indices to the net rate of sodium transport by the skin.

The following symbols represent estimates of variation about the mean of each species.

Species mean, ventral skin, ± 2 Standard Errors

Species mean, dorsal skin, ± 2 Standard Errors

Figure 11

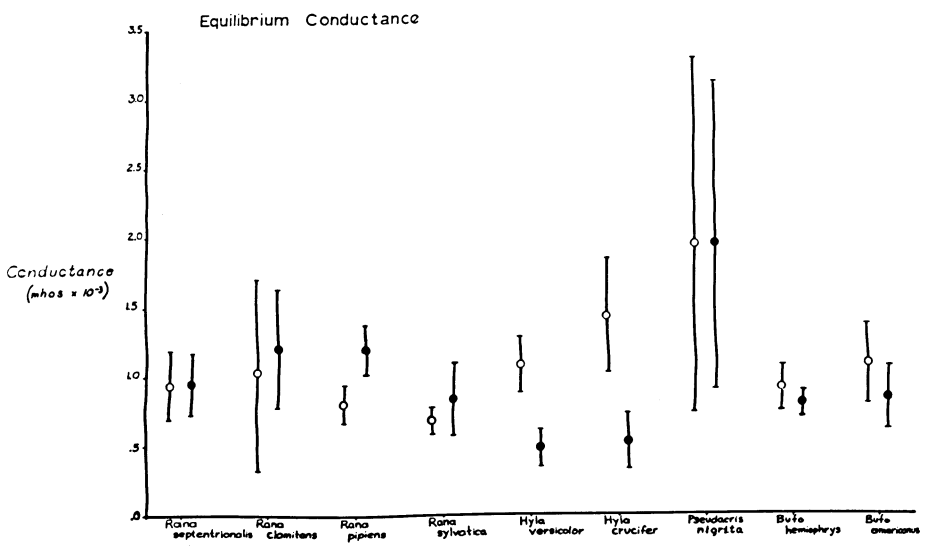


Table 8

EQUILIBRIUM CONDUCTANCE

Species	Mean	Standard Deviation	Standard Error of the Mean	Coefficient of Variation	Number of Preparations
VENTRAL					
Rana septentrionalis	.93	.48	.13	.516	13
Rana clamitans	1.03	.60	.35	.583	3
Rana pipiens	.80	.37	.07	.462	26
Rana sylvatica	.68	.10	.04	.147	4
Hyla versicolor	1.07	.28	.10	.262	8
Hyla crucifer	1.42	.37	.21	.261	3
Pseudacris nigrita	1.94	.96	.68	.495	2
Bufo hemiophrys	.92	.40	.18	.435	5
Bufo americanus	1.09	.32	.14	.294	5
DORSAL					
Rana septentrionalis	.95	.40	.11	.421	12
Rana clamitans	1.20	.36	.21	.300	3
Rana pipiens	1.19	.44	.09	.370	23
Rana sylvatica	.83	.26	.13	.313	4
Hyla versicolor	.48	.20	.06	.417	9
Hyla crucifer	.52	.17	.10	.327	3
Pseudacris nigrita	1.95	.84	.59	.431	2
Bufo hemiophrys	.78	.10	.04	.128	6
Bufo americanus	.85	.30	.11	.353	7

Discussion

Active transport of sodium by amphibian skin does not appear to be an explicit function of the physiological specialization to differences in the availability of water in the preferred habitats of the nine species included in the present study. A second "pathway" in salt conservation which could be of adaptive significance for species in which a high rate of water turnover occurs is in the kidney and bladder. Interspecific difference in the efficiency of the kidney and/or bladder in removing salts from the urine is another possibility which might explain specialization associated with water balance in amphibians. Comparative studies in this area are lacking, and the question of salt conservation being a possible limiting factor in the specialization of anurans to habitats which vary in the availability of water remains incompletely answered.

Response to Controlled Hydration Stress

Materials and Methods

Seventy-one specimens of nine species, R. clamitans, R. septentrionalis, R. pipiens, R. sylvatica, H. crucifer, H. versicolor, B. hemiophrys, B. americanus and P. nigrita, were collected during the summer of 1961. Specimens of R. clamitans were collected near Never's Dam on the St. Croix River. Specimens of H. crucifer and P. nigrita were collected near the Cedar Creek Natural History Area in Isanti County. The rest of the specimens were collected in the vicinity of Lake Itasca. The animals were kept in half-gallon polyethylene jars for not longer than five days. About one fourth of an inch of water and pieces of sphagnum moss were kept in the jars. The animals were not fed.

Each specimen was weighed and measured. The animals were arranged (numbered) in order of decreasing weight within each species sample and separated by placing the odd-numbered animals into the experimental group and the even-numbered animals into the control group.

Specimens of the experimental group were placed in flasks which contained about 300 ml. of distilled water so that only a portion of the head remained above water. As long as the specimen remained alive the water was replaced every other day with fresh distilled water in order to prevent the accumulation of waste products and to maintain the stress of hydration. The flasks were examined every eight hours for dead specimens. Dead animals were

removed and the time of death was recorded as the time of the check period when the specimen was found dead. The animals were not fed during this experiment. An index to the ability of a species to withstand a stress of hydration was obtained by observing the number of days that specimens lived after they had been placed in distilled water.

The specimens of the control group were placed in half-gallon polyethylene jars with loose-fitting lids. About one fourth of an inch of water and pieces of sphagnum moss were kept in these jars. The jars were examined every eight hours for dead specimens. Dead animals were removed and the time of death was recorded as the time of the check period when the specimen was found dead. The control animals were not fed.

Results

The indices of tolerance to a controlled hydration stress, expressed as the number of days of survival after being placed in distilled water, are presented in Figure 12 and Table 9. The results are presented by species, arranged in order from those of more aquatic habitat to those of more terrestrial habitat.

Significant differences were found between the aquatic to semi-aquatic genus Rana and the semi-aquatic to terrestrial genera Hyla and Bufo. The mean of any species of Rana was significantly greater ($P=.05$) than the mean of any species of Hyla or Bufo. The wood frog, Rana sylvatica, appeared to be intermediate in that its mean

Figure 12

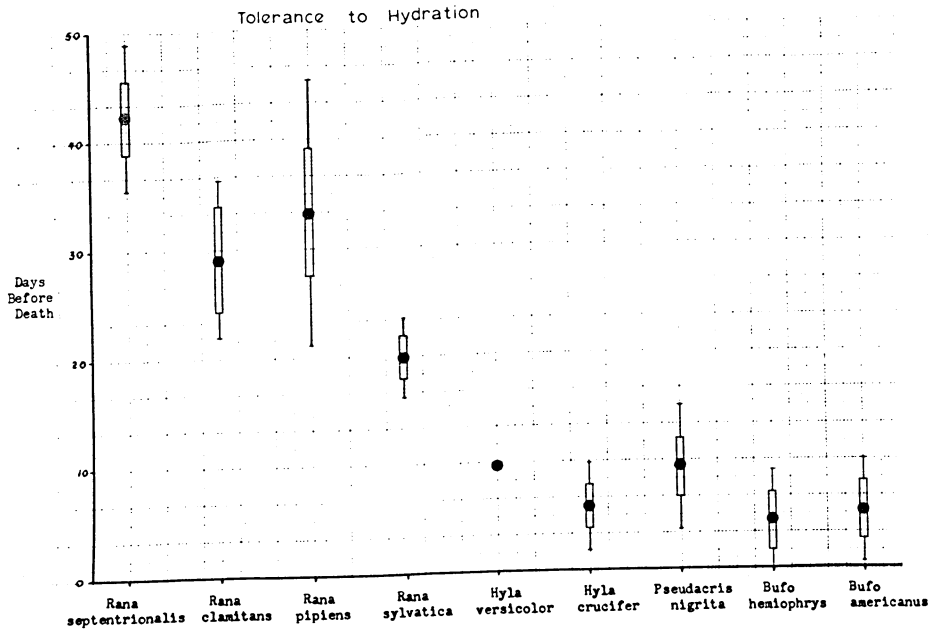


Table 9

TOLERANCE TO HYDRATION
(Days in Water Before Death)

Species	Mean	Standard Deviation	Standard Error of the Mean	Coefficient of Variation	Number of Specimens
Rana septentrionalis	42.5	3.4	1.7	.08	5
Rana clamitans	28.7	3.6	2.5	.12	3
Rana pipiens	33.0	6.6	2.9	.20	5
Rana sylvatica	19.6	1.8	1.0	.09	4
Hyla versicolor	9.3				1
Hyla crucifer	6.7	2.1	.9	.31	5
Pseudacris nigrita	9.1	2.8	1.3	.31	5
Bufo hemiophrys	4.0	2.6	1.3	.64	5
Bufo americanus	5.2	2.3	1.0	.45	6

tolerance was not only significantly different from all species of the genera Bufo and Hyla, but also different from other species of Rana. The values of mean tolerance to hydration stress ranged from 42.5 days for the aquatic mink frog, R. septentrionalis, to 4.0 days for the terrestrial-fossorial Dakota toad, Bufo hemiophrys. The data indicate that there was a tendency for the more aquatic species to be more tolerant to a controlled hydration stress. There was no correlation between the size of the specimen and the tolerance to hydration.

The means, expressed as the number of days of survival, for specimens in the control group are presented in Figure 13 and Table 10. There was no significant difference between the means of any two of the nine species in the control group. The average number of days of survival for animals in the control group was 33.2 days.

There was no significant difference between the mean number of days of survival of animals in the experimental group and those in the control group for the more aquatic species, R. septentrionalis, R. clamitans and R. pipiens. The experimental specimens of the remaining species, R. sylvatica, H. versicolor, H. crucifer, P. nigrita, B. hemiophrys and B. americanus, lived for significantly shorter times, on the average, than did the control specimens. These data suggest that there is a real difference in the abilities of different species of anurans to withstand a controlled hydration stress.

Figure 13

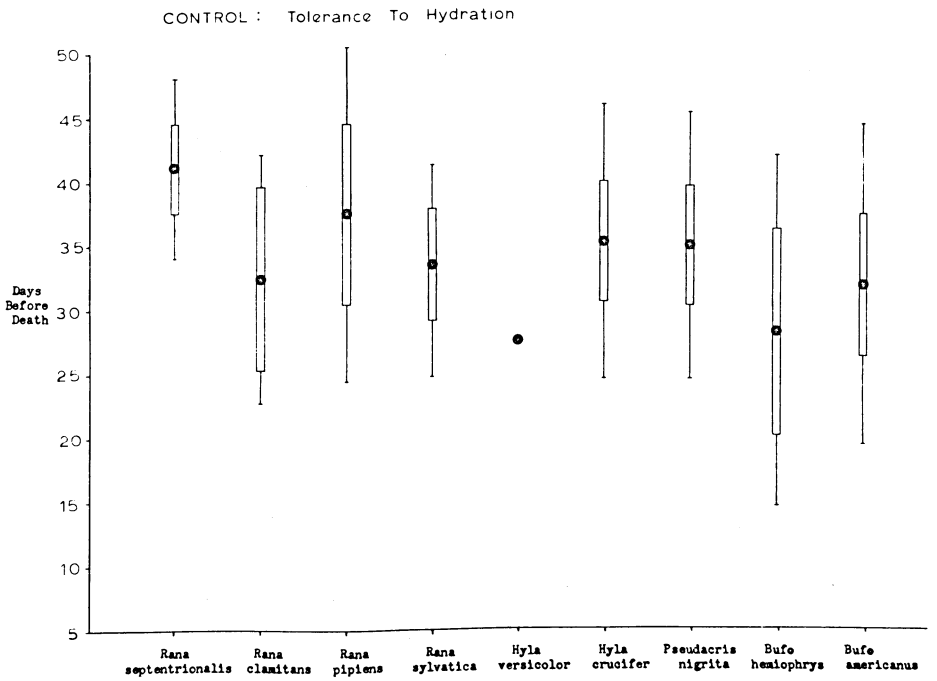


Table 10

CONTROL : TOLERANCE TO HYDRATION

Species	Mean	Standard Deviation	Standard Error of the Mean	Coefficient of Variation	Number of Specimens
Rana septentrionalis	41.1	3.48	1.74	.085	4
Rana clamitans	32.2	4.92	3.48	.153	2
Rana pipiens	37.4	7.17	3.58	.192	4
Rana sylvatica	33.3	3.79	2.19	.113	3
Hyla versicolor	27.1				1
Hyla crucifer	35.0	5.41	2.42	.155	5
Pseudacris nigrita	34.7	5.15	2.31	.148	5
Bufo hemiophrys	28.1	6.81	3.93	.283	3
Bufo americanus	31.7	6.15	2.75	.194	5

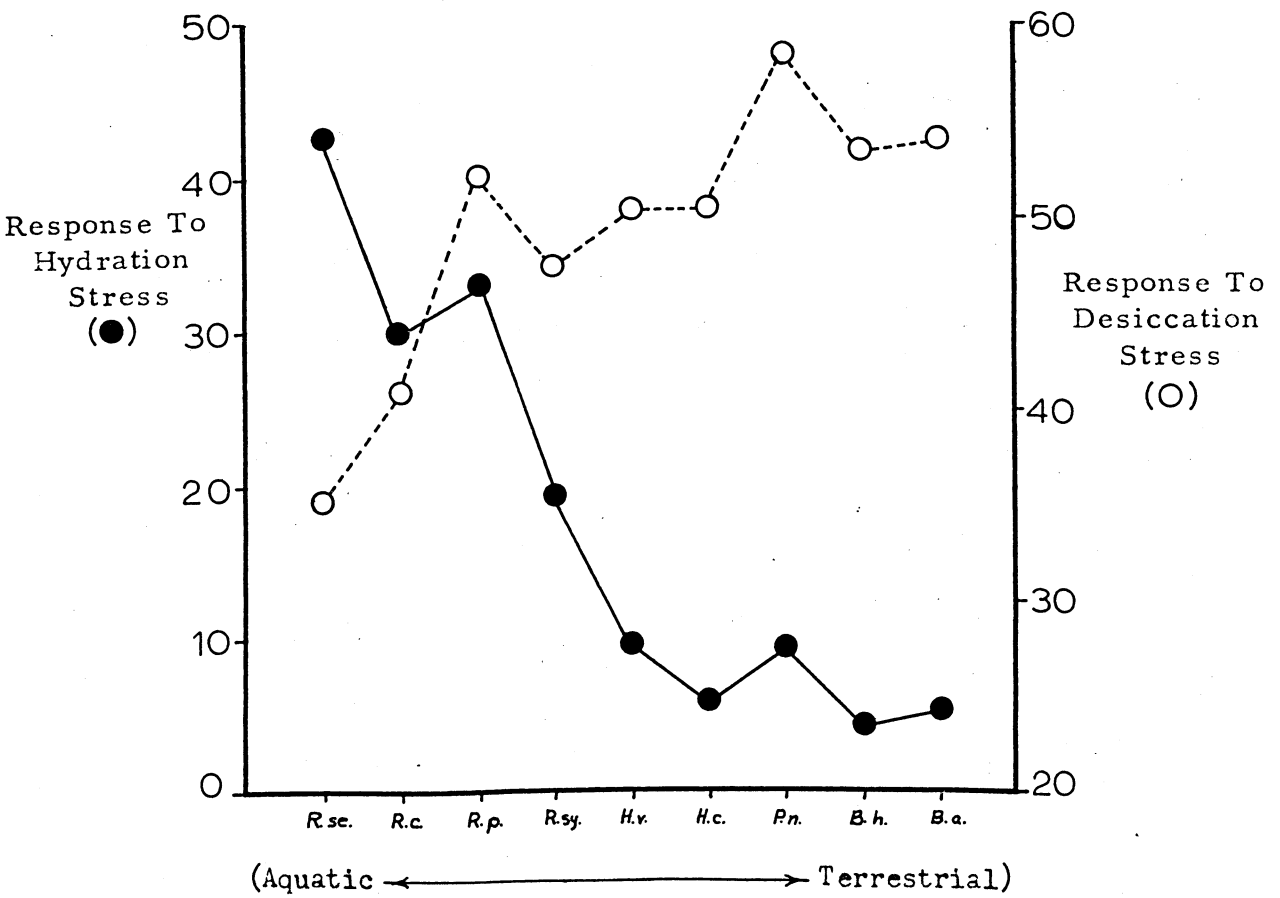
Discussion

The results of these experiments are not only consistent with the results of previous experiments of this study, but also demonstrate interspecific specialization to preferred habitats and provide a basis for predictive statements concerning the natural histories of the species studied with regard to wintering sites.

The significantly lower tolerance to hydration stress could be the result of the more rapid rate at which water entered the bodies of terrestrial species. The results of studies by Rey (1937), Jorgensen (1949, 1950) and Bently, Lee and Main (1958) demonstrated that terrestrial species absorbed water at greater rates than did aquatic species. The tendency for the more terrestrial species to have (i) blood of higher osmolarity and (ii) skin which is more permeable to water may be indicative of the physiological specialization to availability of water which has occurred among amphibians.

Differences in certain aspects of the water economies of nine species have demonstrated specialization to habitats which vary with regard to the availability of water. The more aquatic species remained alive for significantly longer times than did the more terrestrial species. However the terrestrial species tolerated a significantly greater loss of body water when held under desiccation stress. The divergence between response to desiccation and response to hydration stresses for species of different habitat preferences is illustrated in Figure 14. These data suggest that those species which live in terrestrial

Figure 14 Divergence between response to desiccation and response to hydration stresses for species of different habitat preference*



○ Percent water lost before death

● Number of days before death after being placed in water

* Figure 14 constructed from data of Tables 2 and 9.

habitats cannot tolerate aquatic habitats and visa versa. R. pipiens, the species which exhibited relatively high tolerance to both desiccation and hydration stress, has the largest geographical distribution of the nine species studied.

Interspecific differences in response to hydration stress were correlated with differences in habitat preference and wintering sites. The latter correlation is incomplete because of the lack of information on the winter activities of some of the species included in the present study. R. pipiens and R. clamitans, two species which exhibited relatively high tolerance to hydration stress (experimentals were not significantly different from the controls), are known to winter in water. The wintering sites of R. septentrionalis, the third species which tolerated hydration stress, can only be inferred from the indirect evidence that these frogs have been found late in the fall in a lethargic state submerged in water of lakes and streams. R. sylvatica which was intermediate to aquatic and more terrestrial species in response to hydration stress has been observed to emerge from the ground in early spring on the prairie west of Itasca. The toads, B. americanus and B. hemiophrys, are known to spend the winter in the ground, and they have not been reported to winter in water. The correlation between wintering sites and response to hydration might be extended to include those species whose wintering sites are not known. Those species which exhibited tolerance to hydration stress are not able to winter in water. Very little is known

about the winter activities of the tree frogs (Hylidae), but from the results of this experiment it would seem unlikely that they could winter in water. In the late fall of 1960 large numbers of H. crucifer and P. nigrita were observed in sphagnum moss of a tamarack bog near the Cedar Creek Natural History Area. This observation is consistent with the hypothesis that those species of amphibians which were not tolerant to experimental hydration stress cannot spend the winter in water.

Point Summary

1. The water economies of nine species of anurans, including Rana septentrionalis, R. clamitans, R. pipiens, R. sylvatica, Hyla versicolor, H. crucifer, Pseudacris nigrita, Bufo hemiophrys, and B. americanus, have been studied in regard to (i) response to controlled desiccation stress, (ii) effective osmolarity of blood, (iii) permeability of skin to water, (iv) rate of sodium transport by the skin, and (v) response to hydration stress.

2. These nine species were chosen as experimental animals because of availability and wide variation in habitat preference.

3. Species from more aquatic habitats exhibited a lower tolerance to desiccation (percent water lost before death) than species from more terrestrial habitats.

4. Blood plasma of species from more aquatic habitats tended to be of lower effective osmolarity than the blood of species from more terrestrial habitats.

5. Skin of species from more aquatic habitats tended to be less permeable to water than the skin of more terrestrial species.

6. There was no correlation between rates of sodium transport by the skin and habitat preferences of the nine species studied.

7. Species from aquatic habitats were more tolerant to hydration stress than species from more terrestrial habitats.

8. The divergence between response to desiccation stress and response to hydration stress for species of different habitat preferences indicated a specialization among anuran amphibians to the availability of water in the environment.

9. Species from more terrestrial habitats, especially those of the families Bufonidae and Hylidae, may be limited from wintering in water because of this specialization which has resulted in low tolerance to hydration stress.

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